Prevalence of multi-drug-resistant (MDR) bacteria in air samples from indoor and outdoor environments

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ORIGINAL PAPER

Prevalence of multi-drug-resistant (MDR) bacteria in air samples from indoor and outdoor environments

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Abstract The prevalence of multi-drug-resistant (MDR) bacteria in 48 air samples from indoor environments, surgical rooms, dental surgery and waste management plants has been investigated. A total of 280 bacterial strains belonging to different genera were isolated, and the operating rooms were the most contaminated ones (107 isolates), with all the isolates belonging to Gram-positive cocci (51.5 % *Micrococcus* spp., 48.5 % *Staphylococcus* spp.). Only 5 % of the isolates was sensitive to all the antibiotics tested, while the remaining strains resulted resistant to three (13 %), four (14 %), five (9 %) and six (10 %) antibiotics. Correlation between the resistance patterns and the environmental source of MDR bacteria isolates also emerged from the present investigation. This study confirms the high presence of antibiotic-resistant bacteria in air samples, finding that represents a threat for the possible transfer of resistance genes to pathogenic bacteria.

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1 Introduction

An important area of concern for human public health is the incidence of antibiotic resistance among pathogenic and opportunistic bacteria of different sources, and this emerging issue not only represents a nosocomial problem, but also involves a variety of environments (Levy and Marshall 2004). The surveillance of antimicrobial resistance, recently conducted by EARS-Net, has played an important role to provide documentation of the occurrence and spread of this phenomenon, and it constitutes an important source of information on antimicrobial resistance in Europe for policymakers, publics, scientists and doctors. Several reports have emphasized the public health threats of antimicrobial resistance from drug use in humans, animals and agriculture. In particular, hospitals and farms are assumed to be the most significant sources of drug-resistant microorganisms because of their extremely high utilization of antibiotics (Allen et al. 2010; Livermore 2005; Teuber 2001). The indiscriminate use of antibiotics has been at the core of the problem of hospital infections caused by multi-drug-resistant (MDR) bacteria, and considerable debate surrounds the relationship between antimicrobial use

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in animals and the resistance problem in humans (Barza and Gorbach 2002), with the environment as sources of both MDR bacteria and genes encoding resistance. Among environmental matrixes, different authors have studied water and soil for the incidence of MDR bacteria that have been isolated from water and workplace, including food industries and waste management plants. In both cases, few studies have been focused on the role of the air, although it is well known that it represents an important reservoir and vehicle of a great variety of bacteria, becoming crucial in their environmental circulation (Martinez 2009). MDR bacteria and genes encoding resistance, these last acquirable by pathogenic bacteria through genetic recombination mechanisms, frequently have been originated in the environment and can be recovered in outdoor air within more kilometres from the source, which suggests that antibiotic resistance genes may be transported via aerosols on local scales (Davies 1997). Given that a variety of MDR bacteria, like methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and extended spectrum beta-lactamases (ESBL) producing *Enterobacteriaceae*, are responsible both for nosocomial and community infections (Ben-Ami et al. 2009; Livermore 2009; Septimus and Kuper 2009; David and Daum 2010), the presence of airborne MDR bacteria has been investigated to date not only in hospital rooms, but also in other indoor and outdoor environments (Hota 2004; Li and Hou 2003; Tang et al. 2006; Gilbert et al. 2010; Reynolds et al. 2005; Gandara et al. 2006; Lai et al. 2009; Bernard et al. 2012; Vela et al. 2012; Ling et al. 2013; Zhou and Wang 2013).

With the aim to increase knowledge and to make a contribution in monitoring and investigating airborne microbial strains endowed with antibiotic resistance features, we examine the prevalence of MDR bacteria in air samples collected in four types of environments, used for different activities, and then with specific sources of contamination: public, living and working indoor environments, operating rooms, dental surgery and waste management plants. In the isolated species, we investigated the localization of the genes coding for resistance against some antibiotics, since knowledge of the genetic basis is indispensable to define the mechanisms and to determine the speed with this phenomenon can appear and spread within bacterial species and in the environment.

2 Materials and methods

2.1 Sampling and isolation of microbial strains

Forty-eight air samplings were conducted in four types of environments in Modena (Italy), during January–October 2013. These included: (1) indoor environments (16 samples): buildings for local public entertainment as gyms (four samples), restaurants (three samples) and intended for local industrial activities, living and working environments such as homes (three samples), offices (three samples) and classrooms (three samples). All samples were collected during the recreational or work activities; (2) dental practices (six samples): public and private clinics sampled at different times of the day (during activity and at rest); (3) operating rooms (15 samples): different points of the operating room of the Sassuolo Hospital, Departments of Urology and General Surgery, sampled during surgery activities; and (4) solid waste management (11 samples): emptying bins road transport, handling in the landfill and washing of vehicles used for the collection.

Airborne cultivable bacteria were collected with a portable air microbiological sampler. The surface air system (SAS), the viable impactor sampler used in this study (manufactured by PBI International, Milan, Italy), was factory-calibrated, and the sampling head was sterilized (thoroughly wiped with 70 % ethanol) prior to each sampling. Sequential triplicates were taken for each sampling. The sampler was fitted with small-diameter (55-mm) RODAC plates (PBI International) and was operated for 1 min. Plate count agar (PCA) was used for the culture-based sampling, and a pipette was used to fill the 55-mm RODAC plates with 25 ml of medium. Following collection, all culture samples were incubated at 37 °C for 24 h and total colony-forming units (CFU) counted. The most represented bacterial strains were presumptively identified through the observation of the most common macroscopic (colonial appearance, their pigmentation, etc.) and microscopic (morphology of the bacterium, mobility, reaction to the Gram stain, etc.) characteristics. Given the prevalence of Gram-positive species emerged in this study, the isolates that, on the basis of the above evaluations, presented the typical characteristics of the *Micrococcaceae* family were subjected to further investigation, by streaking colonies on Mannitol Salt Agar (bioMerieux, Florence, Italy). Following a 48-h aerobic incubation at 37 °C, presumptive staphylococci were identified

based on colony morphology, Gram staining and slide catalase tests. Gram- and catalase-positive isolates were biochemically identified by Vitek 2 system (GP cards, bioMérieux).

2.2 Antibacterial susceptibility testing

The antibiotic susceptibility was evaluated on 280 strains (mostly belonging to *Micrococcus* and other environmental Gram-positive genera) isolated from 48 air samples of indoor environments, operating rooms, dental surgeries and waste management plants. The minimum inhibitory concentrations (MICs) were determined by the agar dilution method, according to the Clinical Laboratory Standards Institute (CLSI) guidelines 2012. The following antimicrobials were tested: penicillin, ampicillin, amoxicillin, cephalotin, cefuroxime, ciprofloxacin, chloramphenicol, erythromycin, streptomycin, kanamicin, gentamicin, amikacin, tetracycline and rifampicin (all from Sigma Chemical, St. Louis, MO, USA).

2.3 Plasmid DNA analysis

Small-scale plasmid isolation was performed by the rapid mini-prep procedure of O'Sullivan and Klaenhammer (1993) on some randomly selected multi-antibiotic-resistant (MAR) strains. The DNA plasmid was analysed in 0.7 % agarose gel at 3.5 V/cm for 8 h in a Tris–acetate buffer (40 mmol/l Tris–HCl, 20 mmol/l acetic acid, 2 mmol/l Na2 EDTA, pH 8.1). Purified plasmids from *E. coli* V517 were used as the standard markers (Macrina et al. 1978).

2.4 Genotypic characterization

PCR amplification was performed on purified DNA plasmid from erythromycin and tetracycline-resistant *Staphylococcus aureus* strains. Thereafter, specific primers were used to search for genes *erm* (C), *erm* (A), *tet* (K) and *tet* (M) (Strommenger et al. 2003).

3 Results

3.1 Isolation of microbial strains

From 48 air samples examined, a total of 280 bacterial strains belonging to different genera were isolated. The operating rooms were the most contaminated ones (107 isolates), with all the isolates belonging to Gram-positive cocci (51.5 % *Micrococcus* spp, 48.5 % *Staphylococcus* spp). 80.8 % Gram-positive (72.3 and 23.7 % cocci and bacilli) and 19.2 % Gram-negative strains were isolated from all the other environments (71, 68 and 34 isolates from indoor environments, waste management and dental practices, respectively) of which 17.4 % has been ascribed to the genus *Staphylococcus* (with one *S. aureus*-resistant strain isolated), while 82.6 % showed morphological and biochemical characteristics attributable to environmental bacterial genera, generally saprophytes.

3.2 Antibacterial susceptibility testing

For each antibiotic tested, the presence of resistant strains varied widely (Figs. 1a–d). Overall, the resistance to the β-lactam (Fig. 1a) was intermediate, with the only exception of the cefalotin. In particular, 35 % of the strains resulted resistant to penicillin, 30 % to amoxicillin, 38 % to ampicillin, 11 % to cefalotin and 35 % to cefuroxime. The highest levels of resistance were recorded in strains from waste management and hospitals, and intermediate resistance in those isolated from the other indoor environments. The most sensitive strains were isolated from dental practices environment, with the exception of cefuroxime, that showed a 71 % resistance rate, whereas cephalothin was the antibiotic with the lowest levels of resistance (around 11 %), whatever the origin.

Regarding the aminoglycosides (Fig. 1b), the overall rates of resistance resulted very variable. High values of resistance, regardless of the source of the bacteria, were recorded for streptomycin (62 %), intermediate for gentamicin and kanamycin (38 and 43 %, respectively), with an incidence more evident for indoor environments and waste management, and low for amikacin (12 %), in particular for the strains isolated from the operating rooms. With regard to the other antibiotics (Fig. 1c), the strains isolated from hospital environments, indoor environments and waste management showed similar behaviour, with the only difference given by the low resistance to rifampicin in strains of nosocomial source. Relative to the dental environment isolates, the sensitivity varied according to the type of antibiotic, with low resistance values for chloramphenicol and high for the quinolone

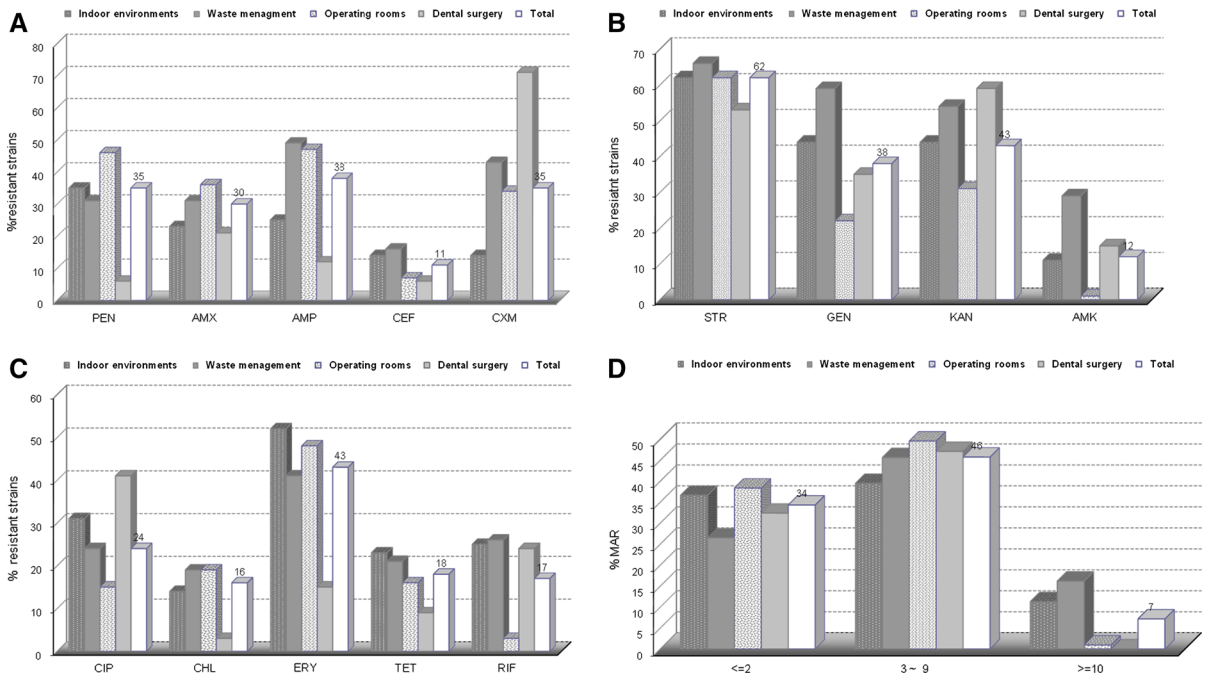

Fig. 1 **a** Incidence of β-lactam resistance in 280 air-collected bacterial strains belonging to different genera. *PEN* penicillin, *AMX* amoxicillin, *AMP* ampicillin, *CEF* cephalothin, *CXM* cefuroxime. **b** Incidence of aminoglycoside resistance in 280 air-collected bacterial strains belonging to different genera. *STR* streptomycin, *GEN* gentamycin, *KAN* kanamycin, *AMK* amikacin. **c** Incidence of other antibiotic resistances in 280 air-collected bacterial strains belonging to different genera. *CIP* ciprofloxacin, *CHL* chloramphenicol, *ERY* erythromycin, *TET* tetracycline, *RIF* rifampicin. **d** Incidence of multiple antibiotic resistances in 280 air-collected bacterial strains belonging to different genera

ciprofloxacin. In particular, 43 % of the strains were resistant to erythromycin, with the lower incidence recovered from the dental practices; for the remaining four antibiotics tested, resistance was low–medium with overall rates of inhibition of 24 % for ciprofloxacin, 16 % for chloramphenicol, 18 % for tetracycline and 17 % for rifampicin. Figure 1d shows the incidence of multiple antibiotic resistances found among the 280 air sampled bacterial strains. Only 5 % of the isolates resulted sensitive to all the antibiotics tested, while the remaining strains resulted resistant to three (13 %), four (14 %), five (9 %) and six (10 %) antibiotics. As expected, the percentages of resistance gradually lowered for higher values of multi-drug resistance with, however, the presence of a 0.4 % of strains resistant to all the antibiotics tested. By grouping multiple resistances in three clusters, 34 % of strains belonged to cluster I (up to 2 resistance), 46 % to cluster II (3–9 resistance) and 7 % to the cluster III (more than 10 resistance). For the first two clusters, the multi-drug resistance rates were similar regardless of their origin, whereas the third cluster showed different behaviours. While the resistance values for the strains isolated from indoor and waste management environments were similar and higher than 10 %, only 1 % of multi-resistant isolates from hospital environments was observed, and none strain from dental practices.

3.3 Plasmid DNA analysis and genotypic characterization

Plasmids, with both high and low molecular weights, were present in 37.5 % of MDR bacteria (strains with 10 or more resistance patterns) and in 100 % of *S. aureus* strains. In particular, three MAR strains (18.75 %) harboured one or two plasmids with low molecular weight, a plasmid with high molecular weight was observed in two MAR strains (12.5 %), while only one MAR strain (6.25 %) showed two plasmids with high molecular weights and four with low molecular weights (data not shown). However, we

did not find any well-defined correlation between the resistance patterns and the plasmid profiles of all the tested strains, as also reported by other authors (Icgen et al. 2002). With regard to the five *S. aureus* isolates, both high (two strains with 2 and 3, respectively) and low molecular weight (all the strains with 8 to 11) plasmids were observed. Figure 2 shows the plasmid profiles of four *S. aureus* strains, two of which resistant to erythromycin and tetracycline. These last resulted also positive for amplification of *erm(C)* and *tet(K)* genes, confirming the extrachromosomal location of their resistance determinants (Figs. 3, 4).

4 Discussion

Although the origins of antibiotic resistance in the environment are relevant to the spread of the genetic determinants responsible for this feature, only a few environmental reservoirs of resistance determinants were thoroughly investigated. In this study, we observed the high presence of antibiotic-resistant bacteria in air samples and a correlation between the resistance patterns and the environmental source of MDR bacteria isolated. Even if for each antibiotic tested the presence of resistant strains varied widely in the different area of sampling, it is clear that waste management and indoor environments might mainly contribute to the spread of to MDR bacteria, strains that could also represent a threat for the possible

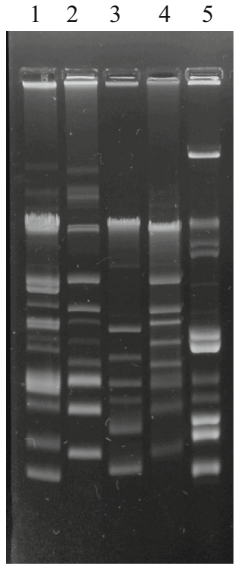

Fig. 2 Plasmid profiles of four *S. aureus* strains. *Lane 1* strain 117, resistant to ciprofloxacin, erythromycin and tetracycline, *lane 2* strain 93A resistant to tetracycline, *lane 3* strain 98D resistant to penicillin G, ampicillin, amoxicillin, streptomycin, gentamicin, kanamicin, erythromycin and tetracycline, *lane 4* strain 99D resistant to tetracycline and streptomycin, *lane 5* molecular size markers prepared from *E. coli* V517 (54, 5.6, 5.1, 3.9, 3.0, 2.7 and 2.1 Kb)

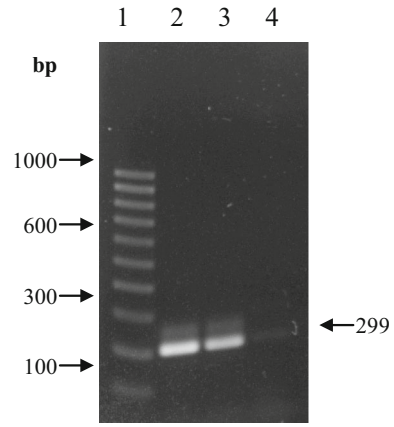

Fig. 3 *erm(C)* PCR gene amplification. *Lane 1* 100-bp DNA ladder, *lanes 2, 3* (strains 117 and 98D, respectively) *erm (C)* PCR gene amplification, *lane 4* negative control

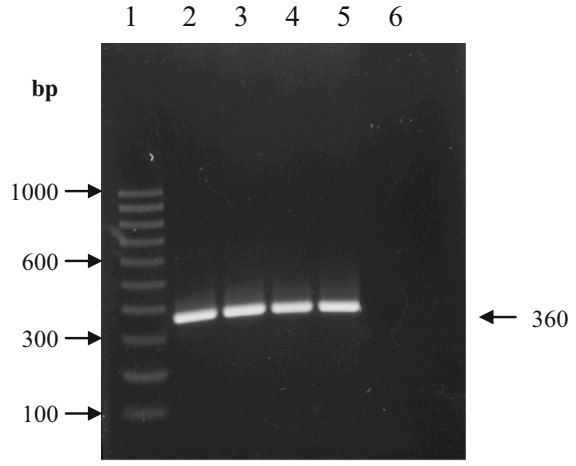

Fig. 4 *tet(K)* PCR gene amplification. *Lane 1* 100-bp DNA ladder, *lanes 2, 3, 4, 5* (strains 117, 99D, 98D, 93A, respectively) *tet(K)* PCR amplification gene, *lane 6* negative control

transfer of the harboured resistance genes to pathogenic bacteria. In particular, two *S. aureus* strains were resistant to erythromycin and tetracycline and resulted positive for amplification of *erm(C)* and *tet(K)* genes. This finding confirms the extrachromosomal location of their resistance determinants, mobile genes capable of crossing bacterial species and, likely, to accelerate dissemination of drug resistance in the environment, posing a threat of a wide diffusion of dangerous bacterial features. From this point of view, our results suggest that air may play a potential role as reservoirs of resistance determinants, prompting the need to undertake epidemiological and molecular studies to

evaluate the mobility of the same. The air collects microorganisms with widely different backgrounds that may have biological characteristics of particular importance for the public health. In fact, the transfer of such microorganisms to humans either directly or indirectly via contamination of objects and surfaces consequently also involves the transfer of important features, such as antibiotic resistance as well as other virulence characters. In fact, the genes for resistance may, at times, be transferred together with those that codify for other important virulence factors (adhesins, gelatinase, biofilm formation, hemolysins, production of substances with antibacterial activity, etc.), a phenomenon that may lead to the appearance of germs with increased virulence and pathogenicity of that observed in past generations (superbugs).

Therefore, it is extremely important to know in time the relationship between the environment and the airborne pathogens before health hazards associated with the relevant pathogens can appear. In fact, while the inappropriate use of antibiotics in humans and animals has led to the emergence of MDR bacteria, the environment has played a decisive role as reservoir of resistance determinants and represents a source for their diffusion among sensible pathogens.

As a result, we strongly assert that surveillance studies should be implemented to better define the role of environmental-resistant bacteria in the maintenance and transfer of drug resistances to other bacteria, including those potentially pathogenic, and its relationship with pathogens in air.

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